FURTHER MEASUREMENTS OF TRYPANOSOMA RHODESIENSE AND T. GAMBIENSE

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In our former paper (May, 1912) on this subject, we came to the conclusion that it was advisable in measuring trypanosomes to confine our observations to those from a single animal, for example, a rat, as although it could not be definitely proved that the size of the trypanosome varied in different animals, yet it appeared likely from the general consensus of opinion that this might be so. From the statistical side the criticism has also been brought that samples of twenty at any particular time are too small. In our present series of measurements therefore, we have, as far as possible, met these objections by measuring always from a single animal, a rat, and by measuring one hundred trypanosomes each day for the first ten days of the infection. We may here briefly repeat our method, as it has been subjected to some criticism.

1. We project the trypanosomes on a screen in a dark room and trace them, instead of drawing them with a camera lucida. It has been objected that this method cannot be used in the wilds of Africa, but we never stated that it could or should, and that is no reason why we should not use it in a laboratory. Our critics might as well object to our using electric light.

2. We measure the trypanosomes by means of the 'tangent line' method. We believe that as this method is the most accurate known—our critics have not attempted to deny this—we are again amply justified in preferring it to the less accurate compass method, even though the difference may be only I or 2μ . It seems to us hardly a matter for argument that if it is worth measuring I,000 trypanosomes at all, it is worth doing so accurately, especially if length is to be considered a criterion of specificity.

We believe that it is important to give the actual data as to measurements as Bruce does, and not simply averages, as the actual data are necessary for a closer analysis than averages permit. We give first, then, the fundamental data for each trypanosome (T. rhodesiense and T. gambiense) and the tables compiled from them. We shall subsequently make a comparative analysis of the two sets of figures.

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6th Day					7th Day					8th Day					9th Day					roth Day					Totals

		Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
1st Day	1 2 3 4 5	30 28 30 29 31	21 19 19 18 22	25.14	26.0 24.05 25.6 24.1 25.95	1.95
2nd Day	6 7 8 9 10	29 29 28 30 26	20 23 16 16 16	22.98	25.65 26.1 21.1 20.85 21.2	5.25
3rd Day	11 12 13 14 15	27 27 30 26 29	19 14 18 19 16	22*51	23·25 22·2 22·85 22·0 22·25	1.25
4th Day	16 17 18 19 20	29 26 26 26 26 28	18 18 16 17 19	22•16	23°0 22°05 20°95 21°2 23°6	2.65
5th Day	21 22 23 24 25	30 28 31 28 34	16 21 19 17 20	24.72	22-8 25-2 25-35 24-75 25-5	2.7
6th Day	26 27 28 29 30	25 27 29 30 29	17 17 18 21 21	24•19	22-0 23·1 24·45 25·85 25·55	3.85
7th day	31 32 33 34 35	22 24 29 28 28	16 17 18 20 17	21•71	19·25 19·9 23·05 23·2 23·15	3:95
8th Day	36 37 38 39 40	32 30 33 33 33 32	15 14 18 20 20	26•15	25°1 24°8 27°75 26°2 26°9	2.95
9th Day	41 42 43 44 45	29 31 32 30 29	17 19 16 22 15	25.67	25:45 25:85 25:5 25:9 25:65	0.42
10th Day	46 47 48 49 50	31 29 31 30 33	18 20 24 19 17	25.98	25.9 25.5 26.95 25.45 26.1	1.42
					Range = 27.75 - 19.25 = 8.5	

TABLE II.—Summary of measurements (in microns) of lengths of 1,000 individuals of Trypanosoma rhodesiense from a single white rat.

							_				
Day	1	2	3	4	5	6	7	8	9	10	Totals
Stumpy 13-21µ	12	4 I	37	45	17	16	53	15	9	8	253
Intermediate 22–24µ	25	19	40	28	31	40	29	18	16	21	267
Long 25-36µ	63	40	23	27	52	44	18	67	75	71	480
	100	100	100	100	100	100	100	100	100	100	1,000

TABLE III.—*T. rhodesiense*, in which the trypanosomes are arranged in Bruce's three groups (a) 13-21µ; (b) 22-24µ; (c) 25µ and upwards.

This table shows very clearly what we have already pointed out, namely, the great variation in the figures for each group on particular days. Thus on the tenth day there were 8 % of stumpy forms, while on the seventh day there were 53 %. This seems to us to make it perfectly obvious that when a sample is taken at random from an animal on any day an erratic factor is introduced.

We proceed to represent the preceding results graphically.

Chart I shows a curve of measurement of Trypanosomarhodesiense. The distribution, by percentages in respect to length, of the 1,000 non-dividing specimens of the trypanosome is plotted. The parasites were taken from the peripheral blood of a rat. One hundred trypanosomes were measured each day for ten consecutive days of infection (vide Table I).



CHART I.—Graphical representation of the distribution of the lengths of 1,000 T. rbodesiense from one rat.

We next proceed to give corresponding tables for T. gambiense.

	of each 100			91.72					26-65					24.14					26.42					23.87	
white rat.	of each 20	22.45	9.12	22.25	26.1	25.4	26.7	26.35	26.92	26.2	27:05	24.3	29.42	22.0	23.4	26.35	26.75	27-25	25-35	27.15	25.6	22.15	24.4	24.75	24.55
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		Maximum	Minimum	Averages of each 100	Averages of each 20	Range of averages of each 20
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2nd Day	6 7 8 9	32 31 30 30 30	22 17 24 22 23	26.65	26.7 26.35 26.95 26.2 27.05	0•85
3rd Day	11 12 13 14 15	29 32 28 28 30	18 17 16 17 21	24.14	24·3 24·65 22·0 23·4 26·35	4.32
4th Day	16 17 18 19 20	36 31 30 32 32	21 21 19 21 21	26.42	26.75 27.25 25.35 27.15 25.6	1.9
5th Day	21 22 23 24 25	26 29 29 31 30	19 16 20 19 18	23.87	22-15 24:4 24:75 24:55 23:5	2.6
6th Day	26 27 28 29 30	32 26 28 28 28 25	20 17 19 17 19	22.88	24·4 22·85 23·35 22·0 21·8	2.6
7th Day	31 32 33 34 35	25 24 26 27 27	16 18 17 16 16	21.07	21.65 21.35 21.15 20.95 20.25	1.4
8th Day	36 37 38 39 40	30 31 31 32 29	20 20 22 21 20	26.37	26:0 25:8 27:0 27:65 25:4	2.25
9th Day	41 42 43 44 45	29 30 32 30 33	21 19 21 18 22	26.17	25.7 26-25 25-65 25-95 27-3	1.65
10th Day	46 47 48 49 50	32 34 32 32 33	19 21 17 17 21	26.94	27.5 27.7 26.55 26.00 26.95	1.7
					Range = $27.7-$ 20.25 = 7.45	

TABLE V.—Summary of measurements (in microns) of lengths of 1,000 individuals of T. gambiense from a single white rat

Day	I	2	3	4	5	6	7	8	9	10	Totals
Stumpy 13-21μ	17	I	25	IO	23	29	57	6	5	9	182
Intermediate 22–24µ	40	16	29	23	33	49	33	15	20	12	270
Long 25-36µ	43	83	46	67	44	22	IO	79	75	79	548
	100	100	100	100	100	100	100	100	100	100	1,000

TABLE VI .- T. gambiense, in which the trypanosomes are arranged in Bruce's three groups.

Here again we note a great variation in the figures for each group, for example, on the second day 1 % of stumpy forms, and on the seventh day 57 %. The differences are due, according to some authors, to a cycle in the vertebrate host.

In Chart II we give a curve representing the distribution, by percentages in respect to length, of the 1,000 non-dividing specimens of *Trypanosoma gambiense*, taken from a rat. One hundred trypanosomes were measured each day for ten consecutive days of infection (*vide* Table IV).



CHART II.—Graphical representation of the distribution of the lengths of 1,000 T. gambiense from one rat.

Comparing now T. gambiense and T. rhodesiense we get the following tables.

	TABLE V	/II												
	Average length Maximum Minimum													
T. gambiense	24·87µ	36·0µ	16°0µ											
T. rbodesiense	24·12µ	34·0µ	1 4 *Ομ											

TABLE VIII .-- Comparison of distribution of the trypanosomes according to Bruce's groups.

		13—21µ	22—24µ	25µ and upwards
T. gambiense	 	18.2 %	27.0 %	54·8 %
T. rhodesiense	 	25.3 %	26-7 %	48.0 %

TABLE IX .- Distribution by Octiles of both T. gambiense and T. rhodesiense.

	125th	250th	375th	500th	625th	750th	875th
T. gambiense	21μ	22μ	24µ	25µ	26µ	27µ	29µ
T. rhodesiensc	20μ	21μ	23µ	24µ	26µ	27µ	28µ

DISCUSSION OF RESULTS

The fact that we are dealing with a dimorphic trypanosome, the dimorphic nature of which is not thoroughly understood, is no doubt responsible for the difference of opinion as to procedure and for the different results obtained. Such difficulties probably do not arise in the case of a monomorphic trypanosome.

I. THE SIZE OF THE SAMPLE

If Tables II and V be examined, it will be found that on one day the average values of five samples of twenty trypanosomes may vary by as much as 4.35μ in the case of *T. gambiense* and by 5.25μ in the case of *T. rhodesiense*. Also the differences between the average value for a sample of 100 on any particular day and any of the samples of 20 for that day may vary by as much as $2\cdot 21\mu$ (third day) in the case of *T. gambiense*, and $3\cdot 12\mu$ (second day) in the case of *T. rhodesiense*, but in the majority of cases it is only about 1μ . From this it may be inferred that a sample of 100 is a fairly reliable one when trypanosomes are plentiful in the blood; it will, however, not be so good a sample as when trypanosomes are scanty.

II. THE INFLUENCE OF THE DAY OF INFECTION

As was indicated clearly in our previous paper, the day of infection in an acute trypanosomiasis (when death ensues in about ten days), such as that with which we have been dealing, is very important in determining whether the trypanosomes are short or long. Thus on examining our results (arranged according to Bruce's groups) in Table VI, we find that stumpy forms vary from 1 % on the second day to 57% on the seventh day, and this variation is, of course, reflected in the average values of 100 for those days, viz., 26.65μ and 21.07μ respectively. Whether these differences occur in so marked a degree in a chronic infection we are not in a position to state.

III. THE INFLUENCE OF THE ANIMAL HOST

As the day is of prime importance, it is impossible to say whether the length of any trypanosome varies markedly in different hosts. So far as we can see, this could only be determined by measuring 1,000 trypanosomes (if this number suffice) from each of the hosts in question. Consequently we consider it is advisable at present to measure from the same species of animal if comparisons are to be of value.

IV. COMPARISON OF OUR RESULTS WITH THOSE OF OTHER OBSERVERS

Curves for *T. rhodesiense* have been constructed by Bruce and his collaborators (1912), by Kinghorn and Yorke (1912), and by ourselves. There is a fair correspondence between those of Bruce and ourselves, but none between those of Kinghorn and Yorke and ourselves. We cannot, unfortunately, in the present state of our knowledge, explain these differences satisfactorily. We believe that they must be due to difference of method, namely, that other observers have taken different species of animals on a variety of days.

We ourselves have now completed three curves (1) based on 1,000 trypanosomes from various animals, but including 600 from rats; (2) based on 600 trypanosomes from rats alone, where samples of twenty were taken on a variety of days from several rats; and now finally (3) 1,000 trypanosomes comprising 100 a day from one rat for ten days. These three curves have this in common, that each one has its main peak at 26μ . Further, the agreement is most close between curves (2) and (3) based on rats alone.

We have given previously our reasons for believing that the third method is the best, and the agreement that exists between our curves indicates, we believe, the consistency of our method. We must admit that Kinghorn and Yorke's three curves are also consistent, but it is noticeable that in their measurements, though different animals were used, the total number of trypanosomes from each animal was the same in each case. There is further the possibility that trypanosomes direct from the natural vertebrate hosts or the fly have a different character from those that have been maintained in laboratory animals.

CONCLUSION

We must admit that we had hoped to be able to distinguish between the two species, T. rhodesiense and T. gambiense, by measuring one thousand specimens of each organism. Though these biometric results are not sufficiently conclusive, we think that it is generally admitted that the two species are distinct.

Microscopically, the two trypanosomes are indistinguishable except by the posterior nuclear character of T. *rhodesiense*. We believe that a curve only expresses graphically what the eye can appreciate under the microscope, and that if two trypanosomes cannot be distinguished microscopically, we shall not be able to do so by measuring them. However, provided that further experience enables observers to agree as to the best procedure, it is no doubt a great advantage to have a correct graphical expression for what is otherwise only an impression, although it may be a quite accurate one. Further, these measurements should not be regarded as useless, as they will undoubtedly form the basis (provided all the protocols are given) for a critical statistical investigation in the future.

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